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Lead article

Pronounced chromosomal instability and multiple gene amplifications characterize ulcerative colitis–associated colorectal carcinomas

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Abstract Patients with ulcerative colitis have a significantly increased lifetime risk for the development of colorectal carcinomas. While genetic and genomic changes during carcinogenesis have been thoroughly studied in sporadic colorectal cancers, less is known about ulcerative colitis–associated colorectal carcinomas. The aim of this study was to extend the identification of specific genomic imbalances to ulcerative colitis–associated colorectal carcinomas and to establish a comprehensive map of DNA gains and losses by investigating 23 tumor specimens from 23 patients. The molecular cytogenetic characterization was performed using comparative genomic hybridization; immunohistochemistry was used to measure proliferative activity and laminin-5 expression as a marker for invasiveness. The results indicate that these tumors are invariably aneuploid, with a high proliferative activity and increased invasive potential. The average number of copy alterations correlates with increased cyclin A levels ($P = 0.044$), which is an independent predictor of risk of carcinoma development in ulcerative colitis. Despite severe genetic instability, the general pattern of specific chromosomal aberrations that defines sporadic colorectal carcinomas is maintained in ulcerative colitis–associated malignancies. High-level copy number increases (amplifications) are dispersed throughout the genome. Strikingly, these amplifications are much more frequent than in sporadic carcinomas and map to chromosomal regions that have not been described before.

1. Introduction

The lifetime risk for the development of colorectal carcinomas is considerably increased in patients with ulcerative colitis. Three in 10 patients will eventually develop cancer after a longstanding colitis [1]. Ulcerative colitis (UC) can therefore be considered a bona fide premalignant condition, and it is recommended that patients with UC participate in surveillance programs to screen for early signs of malignancy. Reliable endoscopic sampling and histopathologic evaluation are difficult, however [2]. A review of 12 surveillance studies with 92 detected carcinomas in 1916 patients revealed that about half of them were advanced Dukes stage C and D malignancies, and only 12% were early carcinomas [3]. It should therefore be obvious that additional markers with high prognostic impact for individual risk assessment are required. As in most carcinomas, tumorigenesis in sporadic colorectal carcinomas is invariably accompanied by the acquisition of specific chromosomal aneuploidies [4]. For instance, the gain of chromosome 7 appears to be an early event in the adenoma–carcinoma sequence, followed by extra copies of chromosome arm 8q, whereas increased copy numbers for chromosomes 13 and 20 occur at later stages of tumorigenesis [4,5]. It has also been established that gross variation in the nuclear DNA content occur prior to the acquisition of mutation in the tumor suppressor gene *TP53* [6]. Of note, increased proliferative activity

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is one of the earliest and most consistent alterations and can already be found in low-grade adenomas [4]. Several studies have reported on nonrandom chromosomal aberrations in ulcerative colitis–associated cancers (UCC) that are more or less similar to those in sporadic tumors [7–11]. The cellular response to chronic inflammation in UC might thus result in genetic instability and the acquisition of specific genomic imbalances.

We report the analysis of 23 cases of UCC by means of comparative genomic hybridization (CGH), measurements of DNA ploidy and proliferative activity as determined by cyclin A expression, and expression of laminin-5. All these parameters were correlated with pertinent clinical features and survival.

2. Materials and methods

2.1. Tumor samples

The study was based on data from 23 patients with UCC. The tumors were diagnosed between 1986 and 2001 at the Department of Surgery, Medical University of Lübeck, Germany. Synchronous UCC could be observed in 5 of the 23 cases. Clinical material was collected from surgically removed tumors, diagnosed on hematoxylin–eosin-stained tissue sections at the Institute of Pathology, Lübeck, Germany, and classified using the tumor–node–metastasis (TNM) classification. The clinical data are summarized in Table 1. Eight sections were prepared from each tumor and used for histologic diagnosis, immunohistochemistry (thickness 4 μm), DNA ploidy measurements (8 μm), and microdissection prior to DNA extraction (50 μm). A second hematoxylin–eosin-stained section was prepared subsequent to the sections for CGH analysis, and the histologic diagnosis was confirmed in all cases. All data were obtained from the dissected areas. Cytogenetic findings were compared with a previously performed analysis of 16 sporadic colorectal carcinomas [4].

2.2. DNA cytometry

Image cytometry was performed on Feulgen-stained histologic sections. The staining procedure, internal standardization and tumor cell selection were based on methods described previously [6]. All DNA values were expressed in relation to the corresponding staining controls, which were given the value 2c to denote normal diploid DNA content. The specimens were divided into two main groups: 1) diploid cases with a distinct peak in the normal 2c region and no cells exceeding 5c and 2) aneuploid cases with a main peak around the 4c region and varying numbers of cells (>5%) exceeding 5c.

2.3. CGH

Formalin-fixed and paraffin-embedded tumor samples were provided in 50-μm-thick tissue sections. The tissue was incubated in xylene (3 × 5 minutes), followed by washes in 95% ethanol. According to the subsequent hematoxylin–eosin sections, the deparaffinized tissue sections were microdissected to obtain representative tissue containing at least 80% of cancer cells. The microdissected samples were placed into Eppendorf tubes with 95% ethanol. After centrifugation, the samples were dried and resuspended in 1 mL sodium isothiocyanate (1 mol/L) and incubated overnight at 37°C. DNA was prepared using high-salt extraction and phenol purification and labeled by nick-translation using biotin-11-dUTP (Boehringer Mannheim, Indianapolis, IN). Genomic DNA from cytogenetically normal individuals was labeled with digoxigenin-12-dUTP (Boehringer Mannheim) as a control. Hybridization was performed on karyotypically normal metaphase chromosomes using an excess of Cot1-DNA (GIBCO BRL, Gaithersburg, MD). The biotin-labeled sequences were visualized with avidin–fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA) and the digoxigenin-labeled sequences were detected with a mouse-derived antibody against digoxigenin followed by a secondary rhodamine-conjugated antimouse antibody (Sigma-Aldrich, Milwaukee, WI). Detailed protocols can be retrieved from http://riedlab.nci.nih.gov. Quantitative fluorescence imaging and CGH analysis were performed using Leica Q-CGH software (Leica Imaging Systems, Cambridge, UK). Interpretation of changes at 1pter and chromosomes 16, 19, and 22 required careful examination, because these loci are prone to artifacts due to the high proportion of repetitive sequences. The CGH results of individual cases, as well as CGH comparison tools, can be found at http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi.

2.4. Immunocytochemistry

All slides were deparaffinized with xylene, rehydrated, and microwaved at 500 W for 2 × 5 minutes in 10 mmol/L citrate buffer, pH 6.0. Intrinsic peroxidase activity was blocked with 3% hydrogen peroxide in methanol, followed by incubation with horse serum (1:20 dilution) in 0.1 mol/L phosphate-buffered saline, pH 6.0. The levels of protein expression were revealed by overnight incubation with the respective antibodies diluted in 1% (weight/volume) bovine serum albumin and visualized with a standard avidin–biotin–peroxidase complex technique (Vector Laboratories). The following antibodies were used (with the respective dilutions and suppliers indicated in parentheses): cyclin A (1:100; Novocastra Laboratories Ltd., Newcastle-upon-Tyne, UK), laminin-5 γ2 chain (1:200, containing amino acids 1017–1178; Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden). Cells were regarded as laminin-5 immunoreactive when a distinct staining of the cytoplasm was visible. The cyclin A immunoreactivity was confined to the cell nuclei. In each specimen, the percentage of stained cells was calculated as reported previously [12]. Based on a semiquantitative scoring system the immunoreactivity for both laminin-5 and cyclin A was

Table 1
Summary of clinical, immunocytochemical, and cytogenetic features analyzed in 23 patients with UCC

Case no.	Sex	Duration of UC (yr)	Age at UCC (yr)	Surveillance	PSC	Synchronous tumors	TNM classification, grading	Histology	Site	Metastasis	Survival (mo)	DNA ploidy	Expression of		CGH amplification
													La-5	Cyc A	
1	M	18	50	Yes	Yes	No	**T4,NX,M1;G3**	AdenoCa	R	Sacrum/liver	15	Aneuploid	None	Low	No
2	M	14	52	Yes	No	No	**T4,N3,MX;G2**	AdenoCa	R-S	Liver	ND	Aneuploid	Increased	Increased	Yes
3	M	10	33	Yes	No	No	**T3,No,MX;G2**	AdenoCa	R	OS sacrum	26	Aneuploid	Increased	Low	No
4	M	15	52	Yes	Yes	No	**T3,N2,MX;G3**	AdenoCa	R	Sacrum/peritoneum	7	Aneuploid	Increased	Increased	Yes
5	F	22	44	Yes	No	No	T3,N2,MX;G3	AdenoCa	T	Ovary/peritoneum	30	Aneuploid	Increased	Increased	No CGH
6	M	22	57	Yes	No	No	**T3,N0,MX;G1**	AdenoCa	Des	—	55[a]	Aneuploid	Increased	Increased	No
7	M	26	65	Yes	No	No	**T1,N0,MX;G2**	AdenoCa	R	—	29	Aneuploid	Increased	Increased	No
8	M	18	60	Yes	No	Yes	**2 × T2,No,MX;G2**	AdenoCa	Des/R	—	37[a]	Aneuploid	Increased	Increased	Yes
9	M	17	38	Yes	Yes	Yes	T1,No,MX;/ **T3,N0,MX;G2**	AdenoCa	Cae	—	29[a]	Aneuploid	ND	ND	Yes
10	M	17	52	Yes	No	No	**T1,No,M0;G3**	AdenoCa	S/R	—	ND	Aneuploid	Increased	Increased	Yes
11	M	25	42	No	No	Yes	T3,No,MX;G1/ T3,N1,MX;G2	ND	Asc/S	Sacrum/peritoneum	33	Aneuploid	low	Increased	No CGH
12	F	10	50	No	No	Yes	2 × T3,No,MX;G2	AdenoCa	S/R	—	63[a]	Aneuploid	Increased	Increased	No CGH
13	M	30	51	No	No	No	**T1,N0,MX;G2**	ND	R	—	2	Aneuploid	Increased	Increased	Yes
14	M	30	54	No	No	No	**T3,N1,MX;G3**	Signet-ring Ca	S	Peritoneum	6	Aneuploid	Low	Low	No
15	F	50	78	No	No	No	**T3,N2,MX;G3**	Signet-ring Ca	R	Sacrum	23	Aneuploid	Low	Increased	No
16	M	30	64	No	No	No	**T3,N0,MX;G1**	AdenoCa	R	—	35	Aneuploid	Increased	Low	No
17	M	10	63	No	No	No	**T3,N3,MX;G3**	AdenoCa	Asc	Liver/peritoneum	9	Aneuploid	Low	None	No
18	M	18	42	No	No	ND	ND	ND	Asc	Liver	5	Aneuploid	Increased	Low	No CGH
19	M	26	37	No	No	No	**T3,N0,MX;G2/3**	AdenoCa	Asc	—	50[a]	Aneuploid	Increased	Increased	No
20	M	20	32	No	No	Yes	T1/Tis/**T2,N1,MX;G3**	AdenoCa	S/T/T	Peritoneum	4	Aneuploid	Increased	Increased	Yes
21	M	11	34	No	No	No	**T3,N0,MX;G2**	AdenoCa	T	—	41[a]	Diploid	ND	ND	No
22	F	59	80	No	No	No	**T3,N1,MX;G2**	AdenoCa	Asc	—	53[a]	Aneuploid	ND	ND	Yes
23	F	33	80	No	No	Yes	T1,N0,MX;G2/ **T3,N0,MX;G3**	AdenoCa	Asc	—	2	Aneuploid	ND	ND	No

Boldface in the TNM/grading column represents tumors included in the CGH analysis.

Abbreviations: Asc, colon ascendens; Ca, carcinoma; Cae, caecum; CGH, comparative genomic hybridization; Cyc A, cyclin A; Des, Colon descendens; F, female; G, grade; La-5, laminin-5; M, male; mo, month; ND, not determined; PSC, primary sclerosing cholangitis; R, rectum; S, colon sigmoideum; T, colon transversum; TNM, tumor node metastasis; UC, ulcerative colitis; UCC, ulcerative colitis associated cancer; yr, year.

[a] Censored.

recorded as follows: category 0, no specific antibody expression; category 1, less than 20%; category 2, 20%–50%; and category 3, greater than 50% immunoreactivity of all mucosal cells. These evaluations were performed by two independent investigators who were unaware of the clinical and histopathologic data. Kappa-values for interobserver variability had been calculated according to low (category 0 or 1) and increased (category 2 or 3) immunoreactivity. Thus, a strong and almost perfect interobserver agreement could be observed for both laminin-5 expression (0.78, 0.81) and cyclin A expression (0.89, 0.90).

2.5. Statistical analyses

To detect the relationships among cytogenetic imbalances, expression levels of immunocytochemical markers, and clinical course, the data were dichotomized and analyzed by Fisher's exact test at a level of $\alpha = 0.05$ using exclusively SPSS version 10.0 (SPSS, Inc., Chicago, IL). Further, Kaplan–Meier survival curves were estimated and compared by a log rank test.

3. Results

We present a comprehensive molecular cytogenetic and phenotypic characterization of 23 cases of UCC. The degree of genomic instability was determined with DNA cytometry and comparative genomic hybridization. Markers for proliferative activity (cyclin A) and invasive potential (laminin-5) were determined with immunocytochemistry. All parameters were compared with tumor stage and grade, clinical features, and patient survival.

3.1. Genomic instability

The TNM staging, tumor grade, histology, and clinical features are summarized in Table 1. All cancers revealed a highly aneuploid distribution of the nuclear DNA content. This pattern was independent of tumor stage. A total of 19 tumors could be analyzed with CGH. Four UCC specimens showed degraded DNA and were thus excluded from CGH analysis. All of the remaining 19 tumors showed chromosomal imbalances as identified with CGH. The most common DNA gains were mapped to chromosomes or chromosome arms 20q (84% of all cases), 7 (74%), 8q (74%), 13q (74%), 11p and 12 (both 42%), 5p and 18p (both 37%), and 17q (31%). Recurrent losses occurred on 8p (58%), 18q (47%), and 5q (26%). High-level copy number increases (amplifications) were dispersed throughout the genome and localized to the following chromosome arms (in decreasing frequency): 8q, 5p, 12p, 13q, 18p, and 20q and also (once each) 4q, 6p, 9p, 10p, 11p, 12q, and 17q. Examples of these amplicons are shown pictorially in Fig. 1. The average number of copy alterations (ANCA), calculated as the number of copy number changes divided by the number of cases, amounted to 13.3 for the autosomes. A summary of genomic imbalances is presented in Fig. 2. A comparative map of genomic

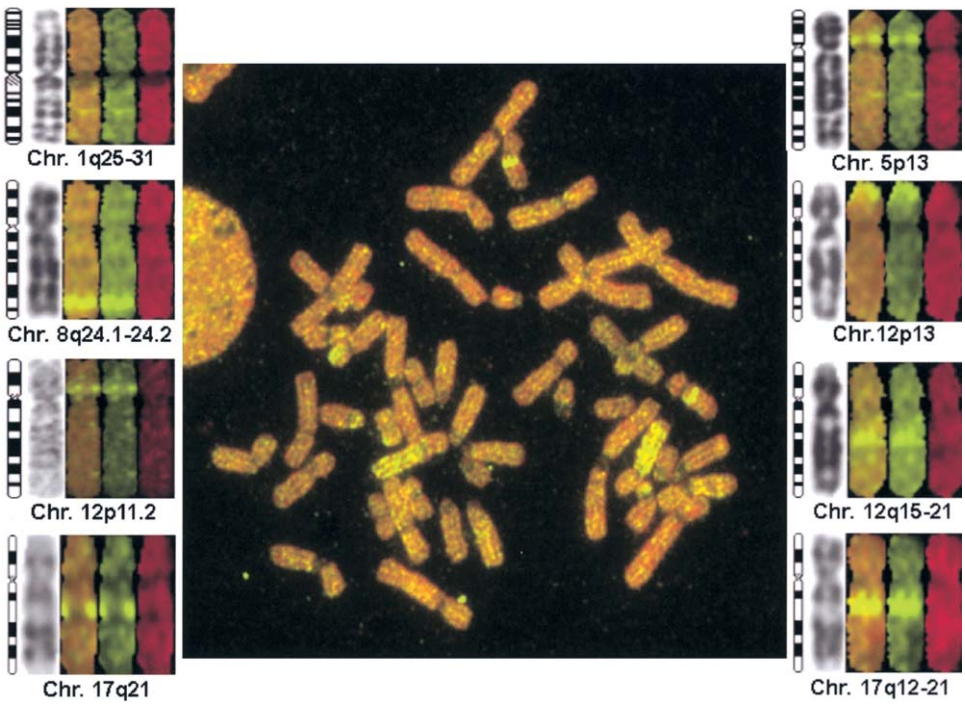

Fig. 1. Examples of distinct genomic aberrations in UCC. Green shows the tumor DNA, and red the control DNA. Both images are combined to calculate fluorescence intensity ratios (in orange). Note the distinct high-level intensities in the green fluorescent channel, indicating significant copy number increases of small genomic regions in the tumor DNA.

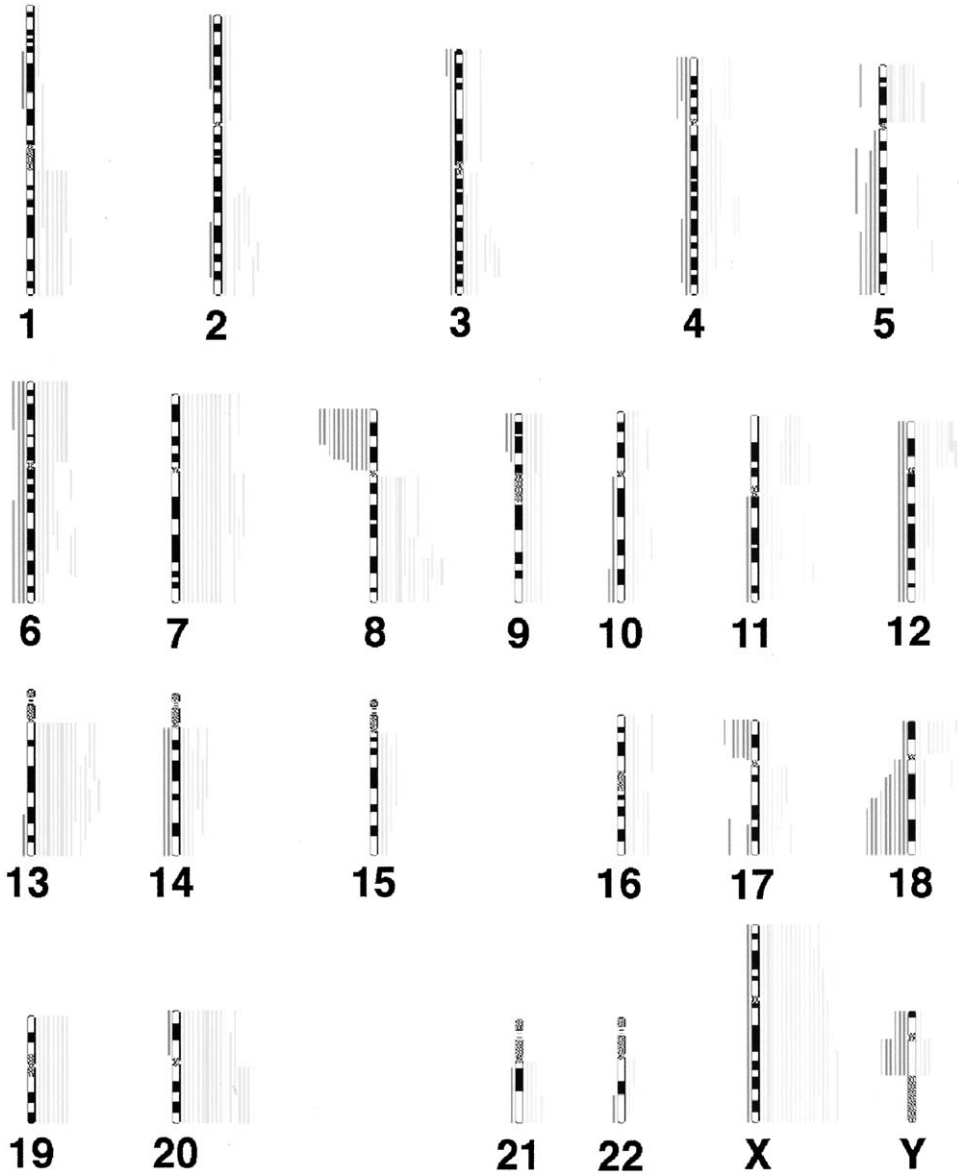

Fig. 2. Summary of genomic imbalances in 19 UCC specimens defined with CGH. Bars on the left side of the chromosome ideogram denote a loss of sequence in the tumor genome, while bars on the right side designate a gain. Bold-face squares or bars indicate high-level copy number increases (amplifications).

imbalances of UCC, sporadic colorectal carcinoma, and primary liver metastasis is presented in Fig. 3.

3.2. Immunocytochemistry

Proliferative activity was analyzed using an antibody against cyclin A. Frequently, proliferative activity is measured with an antibody directed against Ki67, such as Mib1; however, we chose to use an antibody against cyclin A because it allows identification of cells that are committed to pass through the S and G2 phases of the cell cycle [13]. In addition, cyclin A overexpression is associated with poor prognosis in colon cancer, and our own results suggest that cyclin A levels are useful to predict the risk of future carcinogenesis in UC patients [12,14]. We have shown that the majority of the tumors showed increased cyclin A expression levels (i.e., activity in the majority of the cells). Laminin-5 is a marker of tumor invasion in colorectal cancers and an independent predictor of survival in UCC [12]. With the exception of one tumor (case 1), laminin-5 expression was increased.

3.3. Statistical evaluation

To identify predictors of the clinical course, we compared subgroups of patients with respect to survival time, the ANCA value, the presence of amplifications, and proliferative activity as measured by cyclin A levels. High-level copy number increases were correlated with high ANCA values ($P = 0.024$), and both parameters were more commonly observed ($P = 0.044$) in cases with elevated cyclin A levels.

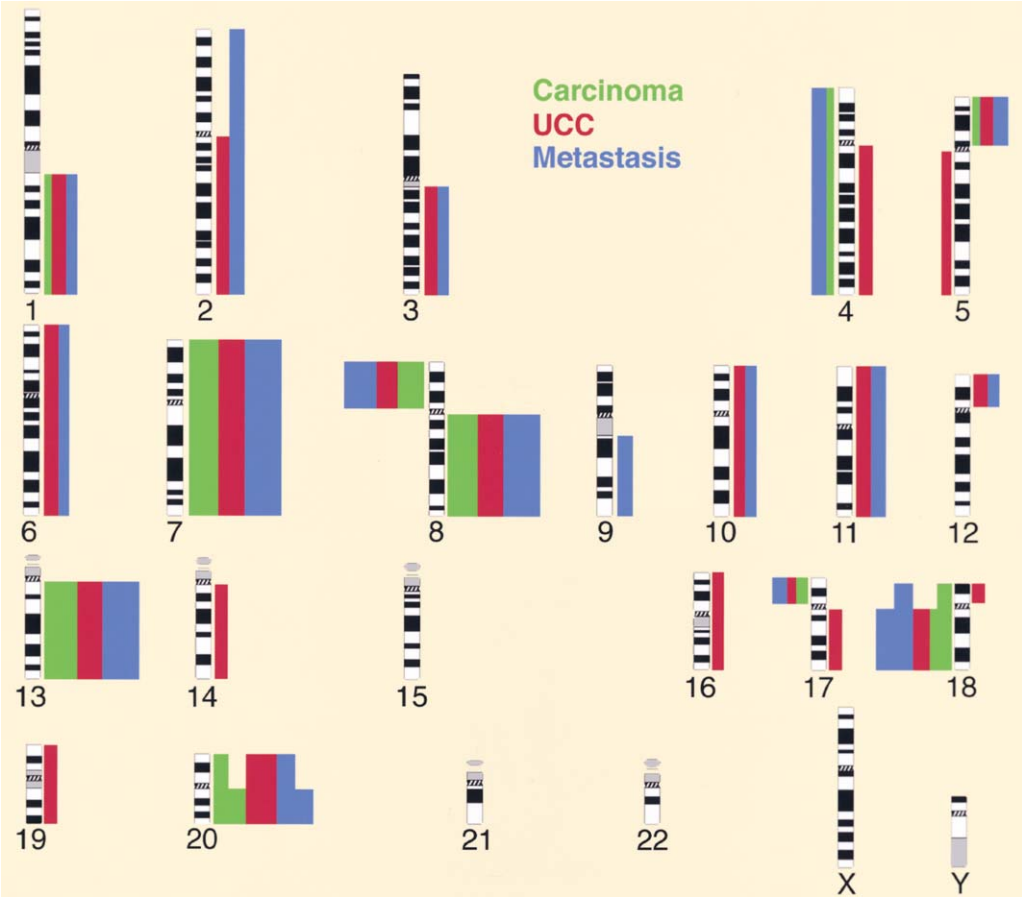

Fig. 3. Comparison of genomic imbalances in sporadic colorectal carcinoma (SCC), UCC, and liver metastasis of SCC. Bars on the left side of the chromosome ideogram denote a loss of sequence in the tumor genome, bars on the right side a gain. The number of alterations per chromosome is normalized to 10 cases for each tumor type. Only ratios greater than 2 have been considered. The SCC group summarized here contains 13 tumors at stage T3, 1 at stage T4, and 2 without staging information. The UCC group consists of 12 tumors at stage T3, 2 at stage T4, 2 at stage T2, and 3 at stage T1.

In addition, all tumors with amplifications were adenocarcinomas and laminin-5 positive; that is, the two cases of signet ring cell carcinomas did not reveal high-level number copy increases. Except for one case, all carcinomas were highly aneuploid and laminin-5 positive. An increased expression of laminin-5 correlated with the presence of metastasis at the time of surgery ($P = 0.045$). Patients with high-grade carcinomas ($P = 0.008$), an advanced TNM stage ($P = 0.03$), and the presence of metastasis at the time of surgery ($P = 0.001$) have a significant shorter survival time (Fig. 4). There was, however, no correlation between survival period and the histology or the location of the tumor, the sex of the patient, the duration of the disease, the presence of primary sclerosing cholangitis, or participation in surveillance programs.

4. Discussion

Patients with UC have a significantly increased lifetime risk for the development of colorectal cancer. Unlike sporadic colorectal tumors, UCCs do not follow the adenoma–carcinoma sequence, and the sequential acquisition of chromosomal aneuploidy and gene mutations is less well established. It is therefore tempting to investigate whether the pattern of chromosomal gains and losses in UCC is similar to that described in sporadic carcinomas. This would indicate that the final distribution of genomic imbalances is the product of continuous selection, and that this distribution is independent of whether a carcinoma occurs spontaneously or as a result of, for example, chronic inflammation.

Several studies have reported a strictly conserved pattern of chromosomal gains and losses in colorectal carcinomas and in primary liver metastases from these tumors. For instance, we could not detect a single metastasis that did not show DNA gains that mapped to at least one of chromosome 7, 8q, 13q, or 20 [15]. More recent reports have provided evidence that, in general, genomic imbalances observed in UCC cluster on the same chromosomes as those in sporadic tumors [7–11]. Our analysis supports these findings; however, we also identified certain significant differences that characterize UCC in contrast to sporadic carcinomas: for instance, the ANCA value was significantly higher in UCC than in stage-matched sporadic colorectal carcinomas [4].

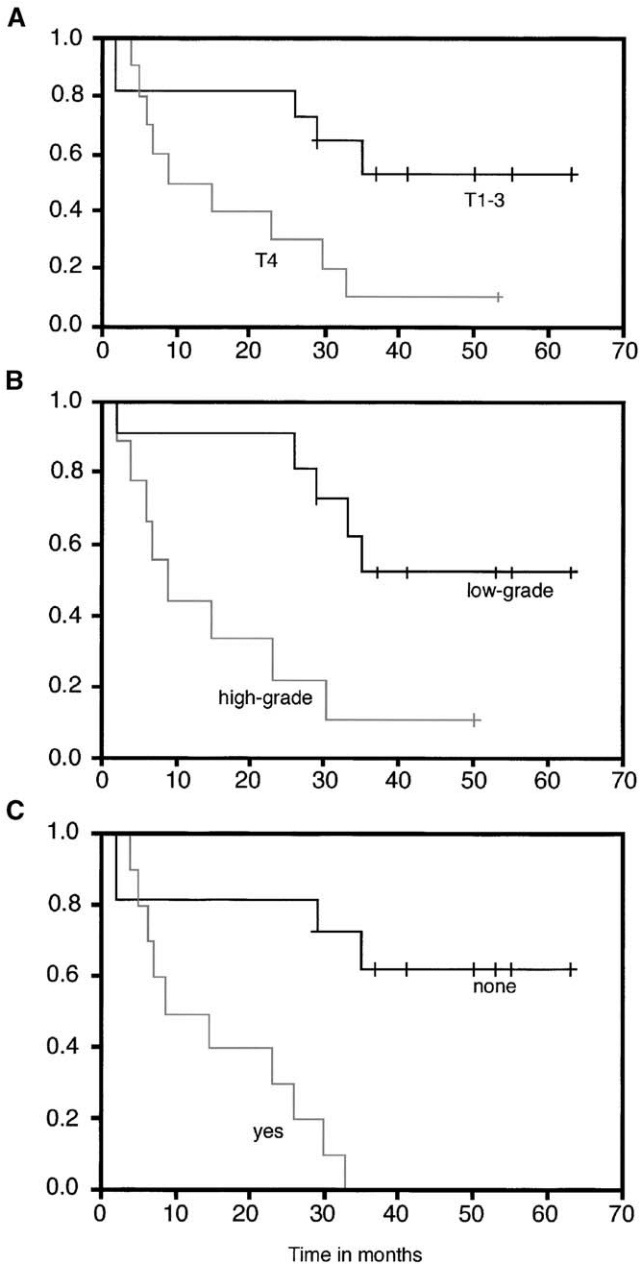

Fig. 4. Kaplan–Meier curves for tumor infiltration as determined by TNM classification (*A*), tumor grading (*B*), and the existence of metastasis (*C*). A crossed line (+) indicates censored observations. *X*-axis: months of survival.

Our previous analyses of sporadic colorectal carcinomas revealed an ANCA value of 5.6, which was elevated to 13.3 in UCC. This number exceeds that observed in primary liver metastases from colorectal carcinomas, for which we had determined the ANCA to be 11.7 [15]. This high degree of genomic instability is also supported by measurements of the nuclear DNA content, which invariably revealed gross aneuploidy.

Additional evidence along this line is provided by our observation of a large number of localized copy number increases. In breast carcinomas, for instance, amplifications are a reflection of advanced disease and correlate with a poorer prognosis [16]. Some of the amplifications occurred in regions known to be affected in colorectal carcinomas, such as chromosome arms 6p, 8q, 13q, 17q, and 20q, and for which the target genes are either known or likely candidates have been identified (http://www.helsinki.fi/cmg/cgh_data.html). For instance, the frequent gain of chromosome 8 and amplifications that map to band 8q24 target the *MYC* oncogene. Candidates on chromosome 20 include the nuclear coreceptor activator gene *NCOA3* [17] and a member of the aurora kinase family [18]; however, amplicons that map to chromosomes 5p (identified three times in our series), 12p (identified 2×), 12q (2×), 9p (1×), 10p (1×), 11p (1×), and 18p (1×) were not known to be associated with colorectal cancers prior to this study. Interestingly, most of these amplifications appear in very distinct locations that allow one to identify possible candidate genes. The amplification on 12q could indicate copy number increases of *MDM2* and could therefore denote an alternative pathway to *TP53* inactivation [19,20]. Also, the 12p amplification could point to an as yet rarely described mechanism of *KRAS* upregulation, whose activation by way of point mutations, but not genomic amplification, has been described in colorectal carcinomas [21]. Another correlation is the coincidental overexpression of laminin-5 and gain of chromosome band 1q25~q31, the mapping position of the *LAMC2* gene (laminin-5). Laminin-5 serves as an independent predictor of cancer risk in UCC [12,22], but it has not been elucidated by which means increased expression levels are produced. Our data suggest that genomic amplification could be one molecular mechanism leading to laminin-5 overexpression. A more detailed characterization of all amplicons is underway.

We have previously analyzed a series of sporadic colorectal carcinomas of comparable clinical stage and grading (Fig. 3). It has therefore become clear that sporadic colorectal carcinomas have fewer genomic imbalances than UCC. The average number of copy alterations in UCC amounts to 13.3. This is a distinctly higher value than that we and others have observed for sporadic colorectal carcinomas [4]. Interestingly, Aust et al. [11] described a much lower ANCA value of 8.6 for UCC; they also found chromosomal losses to be more pronounced than chromosomal gains in UCC (nine affected segments vs. seven). Our study shows a CGH profile in UCC dominated by frequent gains of nine distinct chromosomal locations, compared with only three regions of losses.

Our analysis reports common amplicons on 13 distinct chromosomal bands that have not, to our knowledge, been described before. These differences might be caused by different technical approaches: Aust et al. [11] used tumor DNA amplified by degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR), whereas we used whole genomic DNA without amplification. Arguably, different tissue microdissection procedures would directly influence the percentage of tumor cells. In addition, we could show that the two signet ring cell carcinomas did not reveal the same

pronounced chromosomal alterations as the majority of adenocarcinomas. Aust et al. [11] did not present information about the histopathologic type of the carcinomas analyzed. It might thus be possible that a majority of signet ring cell carcinomas will show a much lower ANCA value than we could establish for UCC. The CGH profile for UCC as presented here, dominated by overall gains and numerous amplifications, is in concordance with the relatively high ANCA value and severe aneuploidy observed in the majority of all 23 UCCs. In comparison, sporadic colon carcinomas show aneuploidy in only 70%–80% of the cases, combined with an overall lower ANCA value. Aust et al. [11] did not present data about the ploidy status of the tissue they investigated.

The surprisingly high level of ANCA values in UCC could be a reflection of a generally increased genetic instability in UCC, due to the long latency of inflammatory disease before overt tumors develop; however, the data presented here and in the literature clearly indicate that the tumor cell population as an entity selects for a distribution of genomic imbalances that is similar to sporadic carcinomas. Therefore, the tissue origin of the tumor cell, and not the mode of tumor induction, defines the similarity between sporadic colorectal cancers and UCC. This is in striking contrast to hereditary colorectal carcinomas arising in the background of mismatch repair deficiency, where neither aneuploidy nor specific chromosomal imbalances are observed [23,24].

The number of cases in this study is, to our knowledge, the largest sample collection from one clinical center that has been reported. The correlation between survival and grading, staging and presence of metastasis is significant (Fig. 4). In contrast, neither high-risk factors for cancer development, such as the diagnosis of a primary sclerosing cholangitis in UC, nor participation in surveillance programs seem to significantly influence a patient's outcome. These findings clearly suggest that additional and more reliable markers for early cancer detection are needed, especially within this subtype of colorectal carcinomas. In this respect, the positive correlation of high ANCA values and the occurrence of amplifications with elevated cyclin A expression indicate a high proliferative activity combined with genomic instability, necessary features for tumor growth and clonal expansion. This finding thus increases the value of cyclin A as an independent prognostic marker for carcinoma development in UC. A positive laminin-5 expression has also been described as an independent marker for cancer risk [12]. The correlation between increased laminin-5 expression and the presence of metastasis at the time of surgery could also point to those patients with elevated laminin-5 levels who are at higher risk for the development of micro- or macrometastases. Potentially, for these patients, treatment options should include adjuvant chemotherapy even in the absence of overt metastatic disease.

Our results suggest that DNA aneuploidy in UCC correlates with specific chromosomal aneuploidies. The pattern is similar to that seen in sporadic colorectal carcinomas. The molecular characterization of the many distinct high-level copy number changes (amplifications) that were not known to be associated with colorectal cancer will likely reveal novel genes involved in colorectal tumorigenesis.

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